ANALYSIS OF SEED LIPIDS AND SYNTHESIS OF FATTY ACID DERIVATIVES

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Dedicated to my beloved papa and parents

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CERTIFICATE

This is to certify that the research work described in this dissertation entitled, "Analysis of seed lipids and synthesis of fatty acid derivatives" has been carried out by Mr. Mohd. Khysar Pasha under our supervision at the Section of Oils & Fats, Department of Chemistry, Aligarh Muslim University, Aligarh. The dissertation is suitable for submission for the award of the degree of Master of Philosophy in Chemistry.

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C O N T E N T S

INTRODUCTION

Many review articles have appeared in recent past dealing with the analysis and structure of natural triacylglycerols. Most of these studies were mainly concentrated to the triacylglycerols having common long-chain fatty acids. But very few studies were made to elucidate the structure of triacylglycerols containing uncommon fatty acids. With the development of new techniques, it is possible to analyze and elucidate the structure of triacylglycerols containing uncommon fatty acids. But the literature in this area is quite scattered. Hence, efforts were made in the present dissertation to compile the literature concerning the structural analysis of triacylglycerols of seed lipids containing uncommon fatty acids.

Oils and fats are widely distributed in nature, mostly in the form of triacylglycerols which abundantly contain common long-chain fatty acids such as lauric, palmitic, stearic, oleic, linoleic, linolenic etc. A large number of uncommon fatty acids such as those containing epoxy, hydroxy, keto, furanoid, cyclopropene, cyclopropane, conjugated olefinic, acetylenic, enynic groups etc. are also known as components of triacylglycerols. This dissertation highlights the work related to structural analysis of triacylglycerols possessing hydroxy, epoxy, acetate, cyclopropenoid and enynic fatty acids.

Chapter I

Review of Literature

Triacylglycerols containing Hydroxy, Epoxy, Acetate and Cyclopropenoic Fatty Acids

Triacylglycerols Containing Hydroxy Acyl Moieties :

Though a large number of seed oils containing hydroxy fatty acids are known, very little information is available concerning the distribution of hydroxy fatty acids in triacylglycerols. In recent years, many seed fats containing hydroxy fatty acids were analyzed by various techniques such as thin-layer chromatography (TLC), column chromatography, gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), ultraviolet spectroscopy (UV), mass spectrometry (MS), lipolysis and various other techniques including chemical methods and radioisotope techniques.

The distribution of ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid in triacylglycerols of well known *Ricinus communis* seed oil (castor oil) was studied by Achaya and co-workers[1,2]. Triacylglycerols of castor oil (containing about 90% ricinoleic acid) consists of 68.2% triricinoleoylglycerol, 28.0% diricinoleoylacylglycerols, 2.9% monoricinoleoyldiacylglycerols and 0.9% of triacylglycerols containing common long-chain acyl moieties. Recently[3] these lipids were re-examined and quantitated by acetylation of the hydroxyl groups of hydroxy acyl moieties by radioactive reagents such as [1-C^{14}] acetic anhydride or [^{3}H] acetic anhydride followed by determination of specific radioactivity of the acetylated lipids. Results obtained by this method were in good agreement with those

obtained by other methods[1,2]. Preferential location of ricinoleoyl moieties at sn-2 position was observed both in monoricinoleoyldiacylglycerols and diricinoleoylacylglycerols by pancreatic lipase hydrolysis. Linum mucronatum[4] seed lipids contain 15% of ricinoleic acid which was found to be present mainly in the form of monoricinoleoyldiacylglycerols.

Gunstone and Qureshi[5] have examined the triacylglycerols of four Strophanthus (Strophanthus sarmentosus, S. courmontii, S. hispidus, S. kombe) seed lipids containing 8-15% isoricinoleic (9-hydroxy-cis-12-octadecenoic) acid. They found that the triacylglycerols containing hydroxy acyl moieties were only composed of monoisoricinoleoyldiacylglycerols, in which the sn-2 position was mainly composed of isoricinoleoyl moieties. Recently Ahmad and co-workers[6] have investigated the distribution pattern of isoricinoleoyl moieties in two Wrightia species, W. tinctoria and W. coccinea which contain 70% and 76% of isoricinoleic acid respectively. W. tinctoria and W. coccinea contained 30% and 38% of triisoricinoleoylglycerol, 42% of diisoricinoleoylacylglycerols, 4% and 2% of monoisoricinoleoyldiacylglycerols, respectively. In the diisoricinoleoylacylglycerols of both seed species the sn-2 position was mainly composed of isoricinoleoyl moieties, while oleoyl and linoleoyl moieties occurred preferentially at the sn-1,3 positions. Equal distribution of isoricinoleoyl moieties between sn-2 and sn-1,3 positions was observed in monoisoricinoleoyldiacylglycerols of W. tinctoria seed lipids.

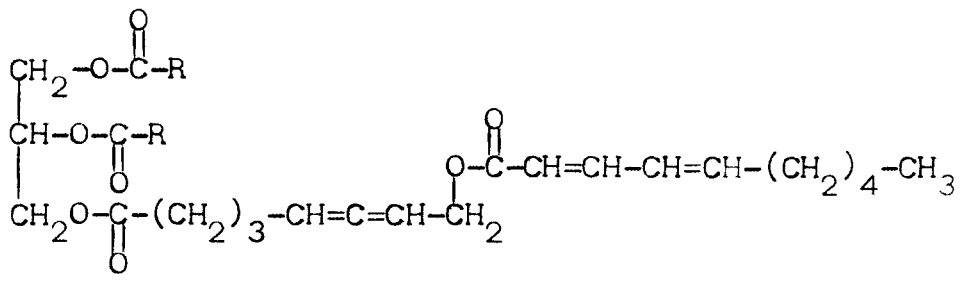
On the other hand, isoricinoleoyl moieties were predominantly esterified at the sn-1,3 positions in monoisoricinoleoyldiacylglycerols of W. coccinea seed lipids. They have also observed the presence of 8 % and 6% isoricinoleoyl moieties in the polar lipids of W. tinctoria and W. coccinea seeds respectively.

Tallent et al.[7] reported the presence of 66% and 68% coriolic (13-hydroxy-cis-9,trans-11-octadecadienoic) acid in the seed lipids of Coriaria nepalensis and Coriaria myrtifolia, respectively. Pancreatic lipase hydrolysis studies showed that the oxygenated unsaturated acyl (corioleoyl) moieties were much more concentrated in the sn-1 and 3 positions rather than sn-2 position.

In all the above mentioned species the hydroxy acyl moieties esterified to glycerol contain free hydroxyl groups. Besides such triacylglycerols, there are several examples in which the hydroxyl group of the hydroxy fatty acids esterified to glycerol are further esterified through an estolide linkage with the carboxylic end of another hydroxy fatty acid or common fatty acid. These types of triacylglycerols are termed as estolide triacylglycerols[8]. The triacylglycerols containing one, two and three estolide bonds are consecutively referred to as monoestolide or tetraacid, diestolide or pentaacid, triestolide or hexaacid triacylglycerols and so on.

Triacylglycerols containing more than three acyl moieties have been identified in several natural oils which contain hydroxy fatty acids. These multiacylglycerols are now expected to be more prevalent in nature than indicated by the literature.

The Sapium sebiferum seed lipids contain two uncommon fatty acids, 8-hydroxy-5,6-octadienoic (ω-hydroxy allenic) acid and trans-2,cis-4-decadienoic acid. These two acids found to be joined by an estolide linkage[9]. In 1969, Christie[10] has separated the S. sebiferum seed lipids by preparative TLC into triacylglycerols containing common long-chain acyl moieties (76.9%) and estolide triacylglycerols (23.1%). The stereospecific analysis of estolide triacylglycerols revealed that the estolide moieties were entirely present at sn-3 position of the glycerol. The trans-2,cis-4-decadienoic acid was found to be totally acylated to the hydroxyl group of the ω-hydroxy allenic acid. The sn-1 position of the normal triacylglycerols was occupied by saturated and monounsaturated fatty acids, whereas the sn-2 and 3 positions abundantly contained linoleic and linolenic acids.

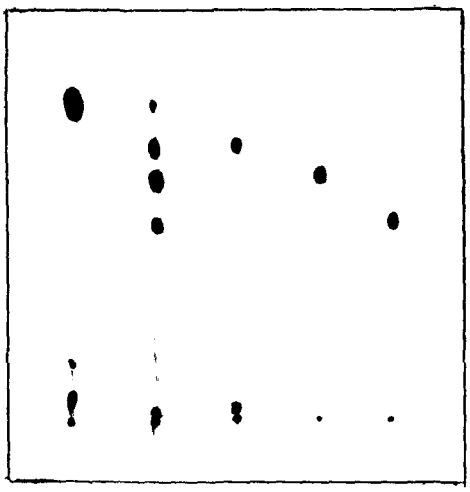


R = $C_{16:0}$; $C_{18:0}$; $C_{18:1}$; $C_{18:2}$; $C_{18:3}$

Monoestolide triacylglycerols of S. sebiferum seed lipids.

In the stereospecific analysis of estolide triacylglycerols, it has been suggested to use pancreatic lipase for the production of diacylglycerols instead of ethyl magnesium bromide, since the later will interfere with estolide bonds present in the triacylglycerol molecules[10-12].

Morris and Hall[13] showed that in ergot (Claviceps purpurea) lipids, ricinoleic (12-hydroxy-cis-9-octadecenoic) acid was esterified with a common long-chain fatty acid. By multiple development TLC with non-polar solvents (5-10% diethyl ether in hexane) they have fractionated the triacylglycerol components of ergot lipids (Fig. 1) and identified them as monoestolide (19.6%), diestolide (22.8%) and triestolide (22.8%) triacylglycerols with the help of TLC by comparing the R_f values with synthetic estolide triacylglycerols prepared from seed lipids of Ricinus communis and by various other methods.



1. Corn oil
2. Ergot oil
3. Monoestolide triacylglycerols
4. Diestolide triacylglycerols
5. Triestolide triacylglycerols

Plate was developed three times with diethyl ether : hexane (7.5:92.5 v/v).

Fig. 1

In the triacylglycerols of ergot lipids the common acyl moieties combined as estolides (Fig. 2) were in general considerably less unsaturated than those attached directly to glycerol and in particular had considerably less linoleic acid. The common acyl moieties of estolides attached to the sn-2 position were in general somewhat more unsaturated than those of estolides attached to the sn-1 and 3 positions of glycerol.

Pancreatic lipase is normally used to determine the fatty acid composition at sn-2 position of triacylglycerols containing a common range of fatty acids or fatty acids with polar or non-polar substituents remote from the ester linkage. Estolide triacylglycerols can also be hydrolyzed by the lipase enzyme but only the glycerol-fatty acid bond is broken while estolide linkage remains intact[10,13-16]. In chemical hydrolysis and transmethylation reactions the cleavage of glycerol ester bond is faster than that of estolide ester bond[13,17]. However, by increasing the reaction period, complete hydrolyses of estolide bonds can be achieved[13].

The triacylglycerols of Lesquerella auriculata[15] seed lipids were found to contain normal as well as monoestolide triacylglycerols (Fig. 3). The normal triacylglycerols (12%) contained none, one and two free hydroxyl groups and so as the monoestolide triacylglycerols (88%). Normal triacylglycerols and monoestolide triacylglycerols containing none, one and two free hydroxyl groups per molecule move together on

silica gel columns. However, they have been effectively resolved by multiple TLC development. In the triacylglycerols of L. auriculata seed lipids, all the estolide moieties were present at sn-1 and 3 positions with most of the saturated long chain acyl moieties. The C_{16} and C_{18} unsaturated acids were found predominantly in the sn-2 position of the glycerol which is a typical characteristic of cruciferous seed lipids[18,19]. All the hydroxy fatty acids (12-hydroxy-cis-9, cis-15-octadecadienoic acid, 14-hydroxy-cis-11,cis-17-eicosadienoic acid and 14-hydroxy-cis-11-eicosenoic acid) of L. auriculata seed lipids were present as estolide moieties in triacylglycerols.

Another species of genus Lesquerella, Heliophila amplexicaulis[20] seed lipids are known to contain 30% of lesquerolic (14-hydroxy-cis-11-eicosenoic) acid. The hydroxy group of lesquerolic acid was further esterified with C_{20} and C_{22} fatty acids (Fig. 4). All the estolide moieties occupied sn-1 and 3 positions and C_{18} unsaturated fatty acids were present at sn-2 position of the glycerol. No estolide moiety was present at sn-2 position.

Phillips and Smith[8] found the estolide triacylglycerols in the seed lipids of Monnina emarginata in which coriolic (13-hydroxy-cis-9,trans-11-octadecadienoic) acid and 13-oxo-trans-9-octadecenoic acid were present along with other common fatty acids (Fig. 5). They have fractionated the seed lipids

into six fractions by double development TLC and identified these as coriolide (14%; lactone of coriolic acid), normal triacylglycerols (18%), monocorioloyldiacylglycerols (27%), triacylglycerols containing one keto acyl moiety (13%), monoestolide triacylglycerols with one keto acyl moiety (0.4%), monoestolide triacylglycerols with one free hydroxy acyl moiety (1.2%) and monoestolide triacylglycerols having common long-chain acyl moieties (37%). Coriolide, which is formed by the acylation of hydroxyl group of coriolic acid had higher R_f value than normal triacylglycerols. It's structure was established by spectral analysis[21]. Estolide and oxygenated acyl moieties in M. emarginata seed lipids exclusively (>97%) esterified at sn-1 and 3 positions, whereas 18:1 and 18:2 acids occupied (>94%) at sn-2 position of glycerol.

Mikolajczak and Smith[16] observed the presence of a trihydroxy fatty acid (9,10,18-trihydroxyoctadec-cis,12-enoic acid) as an estolide moiety in the triacylglycerols of Chamaepeuce afra seed lipids. They have isolated two unusual glyceride fractions containing one free hydroxyl group from C. afra seed lipids. In the first fraction (41.73%), C9-OH and C18-OH groups of trihydroxy fatty acid were esterified with common long-chain acyl moieties, whereas in the second fraction (36.13%), C9-OH and C18-OH groups were esterified with acetyl moiety and common long-chain acyl moieties respectively. The first fraction migrated faster than the second fraction on TLC

plate. Failure of the reaction of triacylglycerols with periodate indicated the absence of free vicinal dihydroxy grouping in the acyl moieties. Treatment with Jones reagent does not yield terminal carboxyl group, which suggested that the C18-OH group was esterified with common long-chain acyl moieties. The acetylated component displayed a strong band at 1230 cm^{-1} in IR spectrum due to acetyl function. Pancreatic lipase hydrolysis of these two fractions revealed that the estolide residues were attached exclusively to sn-2 position of glycerol and common acyl moieties of C_{16} and C_{18} chain lengths occupied the sn-1 and 3 positions.

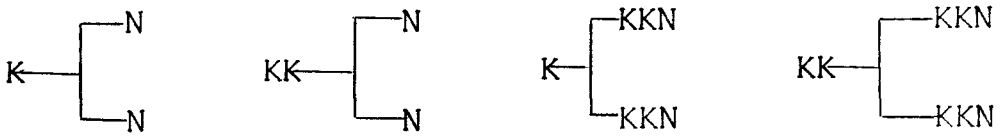
Seed lipids of Nerium oleander and N. indicum[22] contained monoestolide triacylglycerols in which the hydroxyl group of 9-hydroxy-cis-12-octadecenoic acid was found to be acetylated. This unusual component had lower R_f value than normal triacylglycerols. The presence of this unusual lipid moiety was indicated by a conspicuous 1235 cm^{-1} (acetoxy) band in IR spectrum. Its NMR spectrum showed a sharp singlet at δ 1.9, characteristic of an acetoxy group. The 9-acetoxy-cis-12-octadecenoic acid was found to be acylated to sn-1 and 3 positions and $C_{18:1}$ and $C_{18:2}$ to sn-2 position. Mikolajczak and co-workers[23] have reported that 40% of the triacylglycerols of Cardamine impatiens seed lipids contained monoestolide triacylglycerols in which the acetic acid was esterified with one of the two hydroxyl groups of the saturated vicinal

dihydroxy C_{18}, C_{20}, C_{22} and C_{24} fatty acids (Fig. 6). Pancreatic lipase hydrolysis revealed that the estolide moieties were exclusively esterified to sn-1 and 3 positions while C_{16}, C_{18}, C_{20}, C_{22} and C_{24} monoenoic acids occupied sn-2 position of the glycerol moiety.

Seed lipids of Mallotus philippinensis (Kamala) constitute about 72% of ω-hydroxy conjugated trienoic (18-hydroxy-9,11,13-octadecatrienoic) acid which is commonly known as kamalolenic acid. Preliminary examination of the characteristics of the seed lipids was made by O'Neill et al.[24] in 1954. They suggested on the basis of low glycerol content (3.6%) and low hydroxyl value (0.5%) of M. philippinensis seed lipids, that most of the ω-hydroxy groups of kamalolenic acid were esterified. Further, on the basis of the fairly normal saponification value (196) of the lipids, they ruled out the possibility of acetylation and suggested that long-chain acyl moieties were acylated to the hydroxyl group of the ω-hydroxy fatty acid. They also ruled out the lactonization of kamalolenic acid on account of rapid gelling property of seed lipids. The average molecular weight (1130) of total lipids also excluded this possibility.

Achaya and Aggarwal[25,26] suggested that the kamala seed lipids contained four types of triacylglycerols on the basis of high molecular weights, low hydroxyl values, low glycerol contents and the mole ratios of common fatty acids to kamalo-

lenic acid. These were normal triacylglycerols and mono, tetra and pentaestolide triacylglycerols in which kamalolenic acid was either free or esterified with another molecule of kamalolenic acid and/or common fatty acid.



I II III IV

I = Triacylglycerols
II = Monoestolide triacylglycerols
III = Tetraestolide triacylglycerols
IV = Pentaestolide triacylglycerols
K = Kamalolenic acid
N = Common fatty acids

The unequivocal proof for the existence of estolide triacylglycerols in Kamala seed lipids has not been provided to date. No experimental evidence for the presence of diestolide moieties has been given[26]. The lipase hydrolysis has not furnished any evidence of the estolide moiety because of the lack of good experimental conditions. The whole work was carried out with unhydrogenated kamala seed lipids which may affect the analysis because of the rapid polymerizing property[27]

Recently a report[28] appeared describing that in Kamala seed lipids the kamalolenic acid was mainly present as polymeric lactones and non-hydroxy fatty acids were esterified with a pentane diol. The presence of pentane diol has not been proved experimentally. The possibility of the presence of glycerol was ruled out on the basis of the non-formation of tri-p-nitrobenzoyl derivative. If it is assumed that the kamalolenic acid is present in combined form, i.e. as polymeric lactones, then the seed lipids should not possess free hydroxyl groups. Eventually, it has been reported[3] that kamala seed lipids contain about 24.8% of free hydroxyl groups, which could support the glyceride structures proposed by Achaya et al.[20]

The presence of about 40% kamalolenic acid was reported in the seed lipids of Trewia nudiflora L. by Hopkins and Chisholm[29]. Seed lipids of this species were studied for their glyceride structure by Smith and Madrigal[27] and were shown to contain normal triacylglycerols, monoestolide triacylglycerols, diestolide triacylglycerols and triestolide triacylglycerols. Smith and Madrigal[27] observed many difficulties in the lipolysis of hydrogenated T. nudiflora seed lipids because of its high melting point. To overcome these difficulties they have first carried out the lipolysis of unhydrogenated lipids and the lipolysates were then hydrogenated and fractionated by preparative TLC. The acyl moieties released from sn-1 and 3 positions were isolated from lipolysates and

subjected to GC-MS analysis. The presence of estolide moieties was confirmed by comparing their GC-MS data with that of synthetic estolide moieties. All the estolide moieties were reported to be present at sn-1 and 3 positions, but not at sn-2 position.

Triacylglycerols Containing Epoxy Acyl Moieties :

Epoxy fatty acids such as vernolic (12,13 epoxy-9-octadecenoic) acid, coronaric (9,10-epoxy-12-octadecenoic) acid, epoxylinoleic (15,16-epoxy-9,12-octadecadienoic) acid and epoxystearic (9,10-epoxyoctadecanoic) acid are known to occur as acyl components in many natural lipids[30-34]. Even then todate, very little is known about the distribution of these epoxy acyl moieties in triacylglycerols.

A number of workers[35-37] have hydrolyzed the triacylglycerols containing mono, di and trivernoloyl moieties by pancreatic lipase enzyme and reported that the enzyme attacks epoxytriacylglycerols in the conventional manner by preferential cleavage of the acids esterified in the sn-1 and 3 positions. Sampugna et al.[38] reported that vernoloyl moieties were hydrolyzed more slowly than oleoyl moieties.

Isolation and analysis of monoacylglycerols produced from epoxy oils by treatment with pancreatic lipase were complicated since they form chlorohydrins during the course of reaction[38,39]. These complications prompted the development of a new method[35] which involves conversion of free hydroxyl groups of chlorohydrins in the total lipolysates of an oil to trimethylsilyloxy groups and then analysis by GLC. Tallent et al.[36] have fractionated the seed lipids of Euphorbia lagascae, Crepis aurea, and

Cephalaria joppica containing 60%, 56% and 32% of vernoloyl moieties respectively, into nonepoxy triacylglycerols, monovernolins, divernolins and trivernolin by thin layer chromatography. Euphorbia lagascae was found to contain 10% of nonepoxy triacylglycerols, 15% of monovernolins, 56% of divernolins and 19% of trivernolin. On the other hand, no trivernolin was found in the seed lipids of Cephalaria joppica but it was composed of 37% of nonepoxy triacylglycerols, 40% of monovernolins and 22% of divernolins. Seed lipids of Crepis aurea constituted nonepoxy triacylglycerols (13%), monovernolins (18%), divernolins (59%) and trivernolin (10%). The distribution of vernoloyl moieties in mono and divernolins was determined by pancreatic lipase hydrolysis. It was found that more than 80% of the vernoloyl moieties were esterified with sn-1 and 3 positions of the glycerol in monovernolins of E. lagascae seed lipids. Whereas in divernolins, vernoloyl moieties were equally distributed between sn-1, 3 and 2 positions. In the seed lipids of C. aurea and Cephalaria joppica, 61% and 57% of vernoloyl moieties were present at sn-2 position in monovernolin and approximately equal distribution of vernoloyl moieties between sn-1, 3 and 2 positions of glycerol in divernolins was detected.

Vernonia anthelmintica seed lipids are known to contain about 70% of vernolic acid as the component acyl moieties[40]. Before the work of Fioriti et al.[41] on the glyceride studies

of V. anthelmintica seed lipids, the entire vernoloyl moieties were thought to be present in trivernolin form[42-44].

Fioriti and co-workers[41] have suggested that the seed lipids of V. anthelmintica were not only composed of trivernolin but also of di and monovernolins. They have fractionated the V. anthelmintica seed lipids into triacylglycerols containing none, one, two and three vernoloyl moieties by the combination of column and thin layer chromatographic techniques. Pancreatic lipase hydrolysis of these triacylglycerols showed that vernolic acid has marked preference (92%) for the sn-1 and 3 positions in monovernolins, while in the divernolins the sn-2 position was preferred (72%).

About 52% of the vernolic acid constitutes the seed lipids of Erlangea tomentosa[45]. Seed lipids of this species were fractionated by TLC into nonepoxy triacylglycerols (19%), monovernolins (18%), divernolins (47%) and trivernolin (16%). The distribution of vernoloyl moieties in mono and divernolins was determined by pancreatic lipase hydrolysis. In monovernolins, the vernoloyl moieties have shown much preference (84.5%) for sn-1 and 3 positions, while in divernolins sn-2 position was preferred (79.4%). The 1,2 divernoloyl-3-acylglycerols were suggested to be the predominant forms.

A report of Conacher and co-workers[46] appeared in 1970 which describes the glyceride structure of six seed lipids

containing epoxyacyl moieties. The seed lipids of Cephalocroton peuschelli, Cephalocroton cordofanus, Crepis aurea, Crepis vesicaria, Cephalaria joppica and Cephalaria leucantha contain 72, 66.7, 59.6, 52.3, 35.6 and 19.4% of vernoloyl moieties respectively[46]. Though the detailed investigation about the glyceride structure of the seed lipids of Crepis aurea, Euphorbia lagascae and Cephalaria joppica had already been carried out by Tallent and co-workers[36], Conacher et al.[46] re-examined them inorder to compare their results. Seed lipids of C. peuschelli contained nonvernoloyl triacylglycerols (2.8%), monovernolins (13.6%), divernolins (41.2%) and trivernolin (38.8%). Enzymatic deacylation reaction of mono and divernolins indicated that 64.8% of vernoloyl moieties were acylated to sn-1 and 3 positions while 78.7% were found to be acylated with the secondary hydroxyl group of the glycerol moiety. Triacylglycerols of Cephalocroton cordofanus seed lipids were composed of 3.8% nonvernolins, 16.7% of monovernolins, 40.6% of divernolins and 31.8% of trivernolin. Pancreatic lipase hydrolysis of monovernolins revealed that the preference was given by vernoloyl moieties to sn-1 and 3 positions, whereas they were enriched (76.5%) at sn-2 position in divernolins. Cephalaria leucantha seed lipids were found to contain 53.5% of nonvernolins, 30.7% of monovernolins, 9.9% of divernolins and 2.9% of trivernolin. Most of the vernoloyl moieties (74.5%) were acylated at sn-2 position in divernolins and about equal distribution between sn-1, 3 and 2 positions was found in the

case of monovernolins. The nonvernolins (13.5%), monovernolins (14.6%), divernolins (57.5%) and trivernolin (9.9%) constituted the seed lipids of Crepis vesicaria. Vernoloyl moieties (96%) were esterified to sn-2 position of the glycerol in divernolins while they were equally distributed between sn-1, 3 and 2 positions in monovernolins.

Lipase hydrolysis results obtained by Conacher et al.[46] were somewhat different from those reported by Tallent et al.[30] for Crepis aurea and Cephalaria joppica seed lipids. The 2-monoacylglycerols, resulted from lipase catalyzed deacylation, were enriched with 96% of vernoloyl moieties in the divernolins of the seed lipids of above two species.

Triacylglycerols Containing Acetyl (acetate) Moieties :

Acetic acid has long been recognized as a seed lipid constituent[47]. The occurrence of acetotriacylglycerols in the seed lipids of Celastraceae[48], Polygalaceae[49] and Balsiminaceae[50] families is well documented. The first ever report of acetic acid as a component of natural triacylglycerols was made by Kaufmann and Keller[51], who established its presence in the seed lipids of Impatiens roylei.

The seed lipids of Polygala virgata[49] contained 74% of monoacetotriacylglycerols, the first found in nature with the acetate group at sn-2 position of glycerol. Acetotriacylglycerols had low mobility than normal triacylglycerols on TLC plate. Acetotriacylglycerols having acetate group at sn-2 position had higher R_f value than the triacylglycerols having acetate at sn-1 or 3 positions. The presence of acetate group in the triacylglycerols was demonstrated with the help of thin-layer chromatography, infrared spectroscopy and nuclear magnetic resonance spectroscopy.

The NMR spectrum of acetotriacylglycerols of P. virgata seed lipids showed a strong singlet resonance at δ 2.0 attributed to acetate group. The IR spectrum displayed strong absorption band at 1370 cm^{-1} for acetate function. The monoacetotriacylglycerols having acetate group at sn-1 or 3 positions

showed an extra band at 1040 cm^{-1} in IR spectrum which was absent in the monoacetotriacylglycerols having acetate group esterified at sn-2 position[52]. The GLC analysis of the hydrogenated monoacetotriacylglycerols of P. virgata seed lipids showed six molecular species of the following composition $C_2C_{14}C_{14}$, 3.9%; $C_2C_{14}C_{16}$, 1.9%; $C_2C_{16}C_{16}$, 10.5%; $C_2C_{16}C_{18}$, 3.9%; $C_2C_{18}C_{18}$, 22.6%; $C_2C_{18}C_{20}$, 3.3%. The acetate group was suggested to be present at sn-2 position since the monoacetotriacylglycerols were optically inactive. This was further supported by comparing the R_f value with the authentic 2-acetotriacylglycerols and by NMR studies using shift reagents.

Smith et al.[49] have envisaged difficulties during the hydrolysis of acetotriacylglycerols with pancreatic lipase. They could not isolate 2-monoacetylglycerol because of its high solubility in aqueous phase. However, they were able to detect the glycerol. On the basis of this observation, they have suggested that 2-monoacetylglycerol was hydrolyzed rapidly in the aqueous phase.

Kleiman et al.[48] have investigated the seed lipids of Euonymus verrucosus, which was found to contain more than 90% 1,2-diacyl-3-acetylglycerols. The characterization of 1,2-diacyl-3-acetylglycerols was made by thin-layer chromatography, gas liquid chromatography, infrared spectroscopy, optical rotatory dispersion (ORD) and lipolysis. Lipase hydrolysis of

1,2-diacyl-3-acetylglycerols yielded two types of diacylglycerols, one containing common long-chain fatty acids and another containing acetic acid and common long-chain fatty acids. The monoacetotriacylglycerols traversed slower than triacylglycerols containing common long-chain fatty acids on TLC plates. The presence of acetate group in the acetotriacylglycerols of E. verrucosus at sn-1 or 3 positions was also established on the basis of ORD studies. Acetotriacylglycerols exhibited same optical rotatory dispersion as that of synthetic 1,2-diacyl-3-acetylglycerols. No acetate group was found to occupy the sn-2 position since no component having R_f value equal to 1,3-diacyl-2-acetylglycerols was detected on TLC plates. Further 1,3-diacyl-2-acetylglycerols did not show optical rotatory dispersion. The resolution of monoacetotriacylglycerols according to carbon number was achieved by GLC, which showed only two peaks corresponding to C_{36} and C_{38}. The presence of monoacetotriacylglycerols (13-98%) in seven other species of Celastraceae and five species in three other plant families was also determined by Kleiman et al.[48] with the help of IR, NMR, TLC, and GLC techniques. Monoacetotriacylglycerols containing acetate group at sn-1 or 3 positions were also reported to be present in the seed lipids of Impatiens edgeworthii[50].

Triacylglycerols Containing Cyclopropene Acyl Moieties :

Cyclopropene fatty acids have been found principally in seed lipids, though they also exist in other tissues of four plant families of the order Malvales (Sterculiaceae, Malvaceae, Bombacaceae and Tiliaceae) and may be accompanied by small amounts of the saturated analogues of sterculic and malvalic acids (dihydrosterculic and dihydromalvalic acids). In the seed lipids, cyclopropene fatty acids (CPFA) such as sterculic (9,10-methylene-9-octadecenoic) and malvalic (8,9-methylene-8-heptadecenoic) exist largely as triacylglycerol components, though they have also been found in the phospholipids[53,54].

The cyclopropene fatty acids showed numerous physiological disorders[55-57] which were attributed to the highly strained three membered unsaturated ring structure. The sterculic acid exhibited more carcinogenic induction than malvaloyl moieties[58]. Because of these unusual physiological properties of CPFA, they have attracted much attention in recent years. The CPFA moieties were also found in certain edible fats[55].

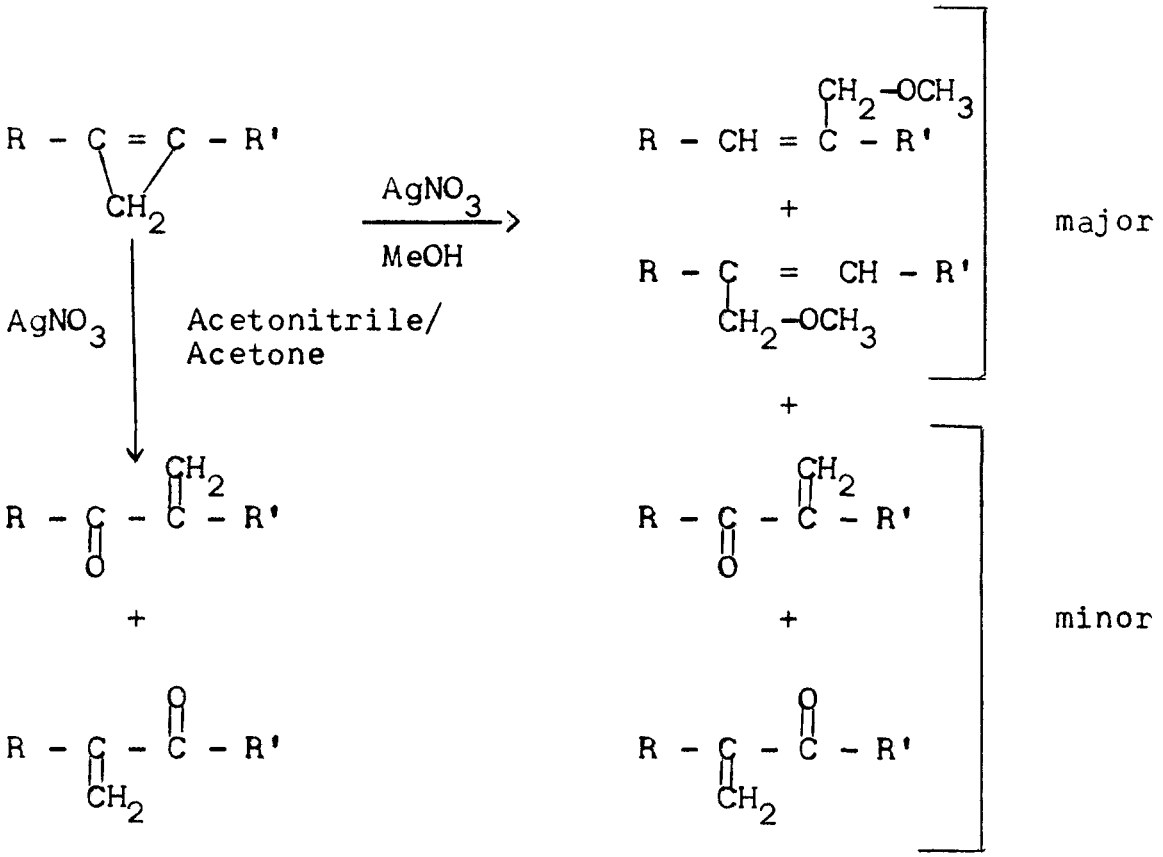
The structure of triacylglycerols containing CPFA moieties has received very little attention, despite the occurrence of these substances in edible fats and despite their biological significance. The principal reason for this was the presence of the highly reactive cyclopropene ring. Direct gas liquid chromatography (GLC) of intact triacylglycerols containing CPFA

moieties resulted in rapid thermal polymerization at high temperature. The cyclopropene groups when treated with silver nitrate produced different derivatives depending on the organic solvent used[59-61]. Consequently, separation of triacylglycerols containing CPFA moieties into fractions differing in the number of olefinic bonds cannot be accomplished by argentation thin-layer chromatography (TLC). It was reported that the CPFA moieties may partially inhibit lipolysis by pancreatic lipase which raises problems if the positional distribution of such acyl moieties in the triacylglycerol molecule is to be determined by lipolysis[62].

Earlier, attempts were failed to successfully resolve these triacylglycerols by GLC after converting the CPFA moieties into the more stable cyclopropane[63] and mercaptoderivatives[64]. A number of methods have been reported[65] for the analysis of fatty acid methyl esters containing CPFA moieties including the use of well conditioned glass column packed with non-polar stationary phase at low temperature (150°C)[66,67] and hydrogenation of cyclopropene moieties to cyclopropane moieties using heterogeneous[68,69] and homogeneous[70] catalysts followed by GLC analysis. But none of these methods can be used for the analysis of triacylglycerols containing CPFA moieties into individual molecular species.

The cyclopropene groups react quantitatively with silver

nitrate to produce α,β-unsaturated ketones, in the presence of non-hydroxylic solvents such as acetonitrile and acetone[59]. In the presence of methanol, this reaction produces methyl ethers as major and α,β-unsaturated ketones as minor products[60,61] as shown below :



R = $CH_3-(CH_2)_7$

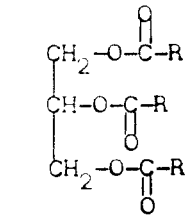
R' = $HOOC-(CH_2)_7/HOOC-(CH_2)_6$

Schuch and co-workers[71] have recently analyzed the triacylglycerols of Bombax munguba seed lipids which contain

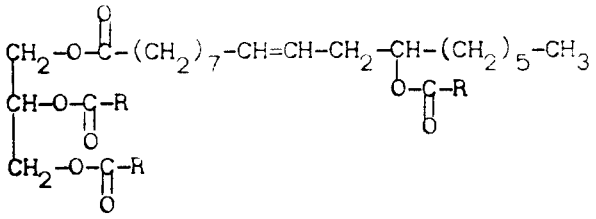
30% CPFA. They have treated the pure triacylglycerols of B. munguba with silver nitrate in acetonitrile and acetone (1:1, v/v) in order to convert the cyclopropene groups into thermally stable and easily separable α,β-unsaturated keto derivatives. They have fractionated the derivatized triacylglycerols by preparative TLC into molecular species containing none, one and two keto acyl moieties (corresponding to none, one and two cyclopropene acyl moieties) per molecule. The fatty acid composition of each molecular species revealed that the triacylglycerols of B. munguba were composed of three types of molecular species. The molecular species containing none, one and two cyclopropene acyl moieties per molecule constituted, respectively, 41%, 50% and 9% in the total triacylglycerols. They have also analyzed the triacylglycerols containing no CPFA and one CPFA moiety by gas chromatography according to carbon number. The triacylglycerols containing no CPFA moieties were resolved into tripalmitoylglycerol (12.3%); 1,3-dipalmitoyl-2-oleoylglycerol and 1,3-dipalmitoyl-2-linoleoylglycerol (66.5%). The triacylglycerols containing one CPFA moiety were composed of 1,3-dipalmitoyl-2-sterculoylglycerol (87.0%) and 1,3-palmitoylstearoyl-2-sterculoylglycerol (13.0%). It was found that the sterculoyl and malvaloyl moieties were predominant at sn-2 position of the triacylglycerols.

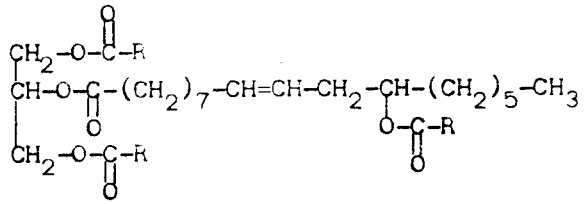
We have recently analyzed the triacylglycerols of Sterculia foetida[72] seed lipids containing about 72% of CPFA

by adapting the method reported by Schuch *et al.*[71] The triacylglycerols of *S. foetida* seed lipids were fractionated into four (I-IV) types of molecular species differing in the number of keto acyl moieties (corresponding to number of CPFA moieties). The molecular species II (41%) contained one CPFA moiety, molecular species III (33%) had two CPFA moieties, and IV (20%) had three CPFA moieties. A minor molecular species I (6 %) contained only common long-chain acyl moieties. Pancreatic lipase hydrolysis revealed that the oleoyl and linoleoyl moieties were preferentially esterified at *sn*-2 position of glycerol while palmitoyl moieties were abundantly located at the *sn*-1,3 positions. The sterculoyl moieties showed preference for the *sn*-2 position and the malvaloyl moieties for *sn*-1,3 positions.

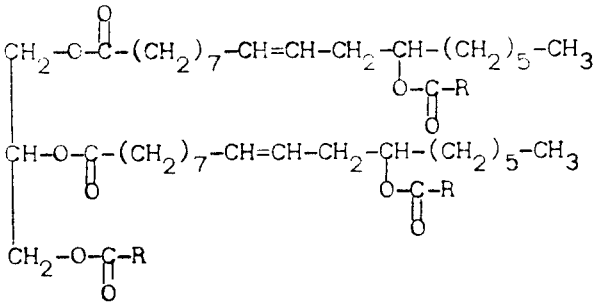


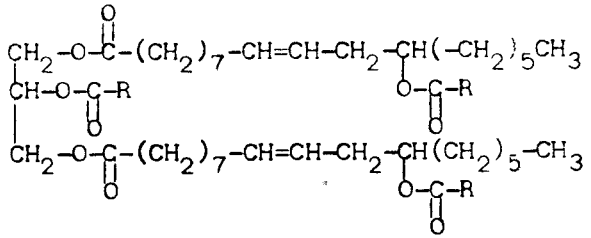
Normal
Triacylglycerols



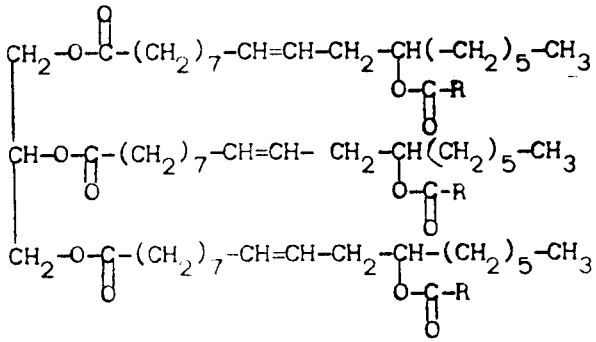


Monoestolide triacylglycerols





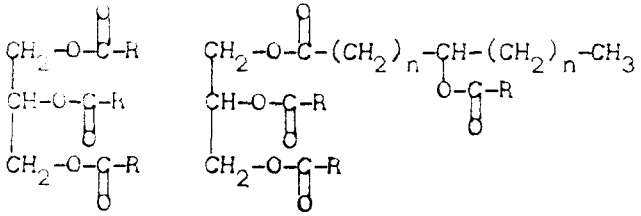
Diestolide triacylglycerols



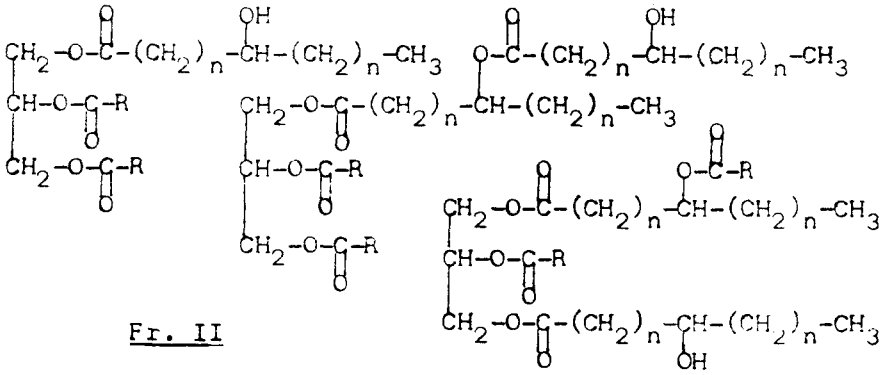
Triestolide triacylglycerols

R = C_{14}, C_{16} and C_{18} acyl moieties.

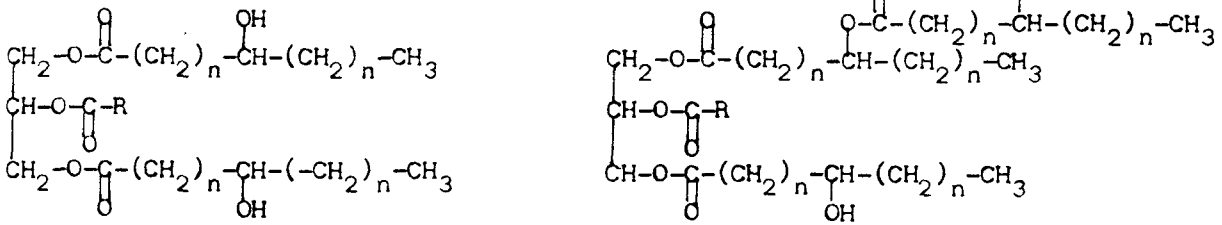
Fig. 2: Schematic representation of the structure of triacylglycerols present in ergot lipids



Fr. I



Fr. II

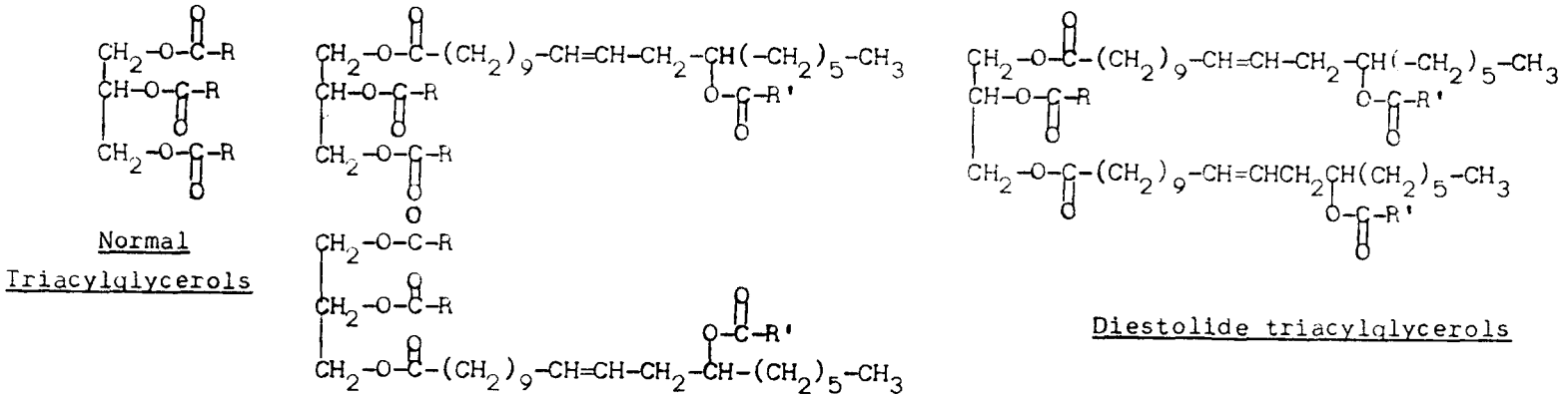


Fr. III

R = 14:0; 16:0; 16:1; 18:1; 18:2; and 18:3 acyl moieties.

Hydroxy fatty acids = 18:1-OH; 18:2-OH; 20:1-OH and 20:2-OH acyl moieties.

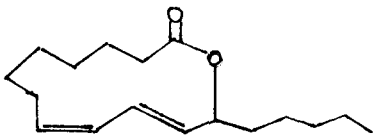
Fig. 3: Schematic representation of triacylglycerols in Lesquerella auriculata seed lipids



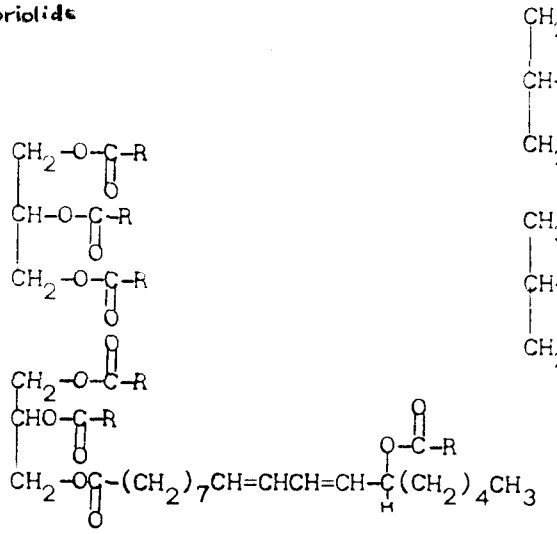
R = 14:0; 16:0; 16:1; 18:0; 18:1; 18:2 and 18:3 acyl moieties.

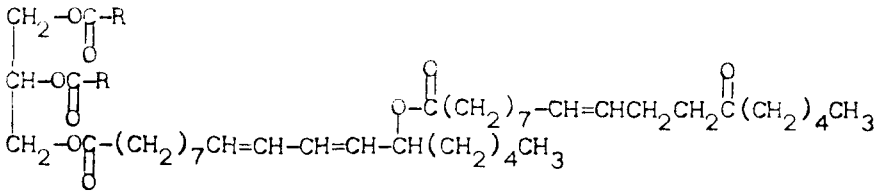
R' = 20:0; 20:1; 22:0; 22:1; and 22:2; acyl moieties.

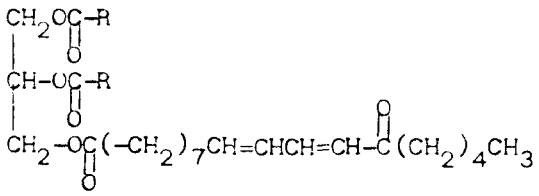
Fig. 4: Schematic representation of triacylglycerols present in Heliophila amplexicaulis seed lipids.

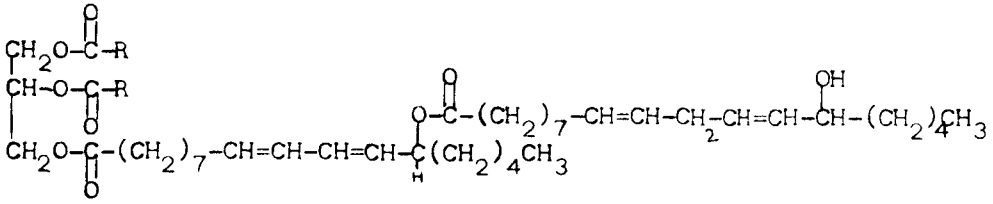


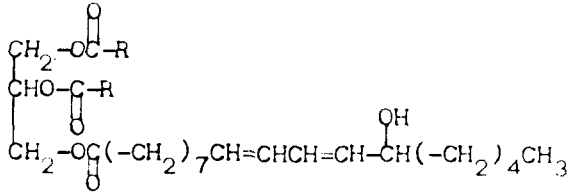
Coriolide





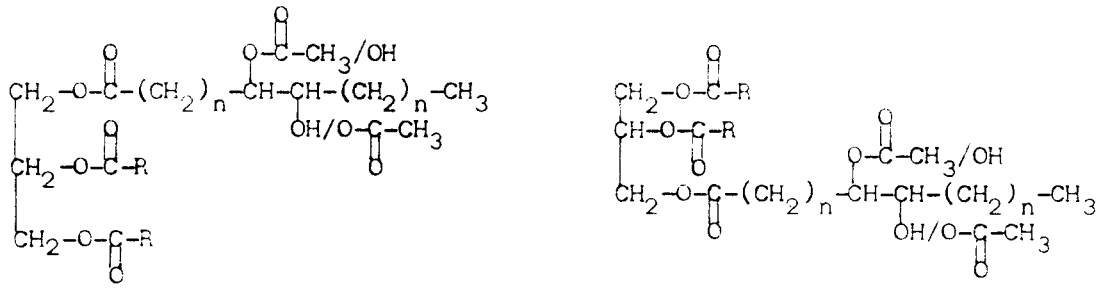






R = C_{16} and C_{18} acyl moieties.

Fig. 5: Schematic representation of triacylglycerols in Monnina emarginata seed lipids



Dihydroxy fatty acids : C_{18}, C_{20}, C_{22} and C_{24}

R = C_{16}, C_{20}, C_{22} and C_{24} acyl moieties.

Fig. 6: Schematic representation of the monoestolide triacylglycerols of Cardamine impatiens seed lipids

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Chapter II

Present Work

Analysis of Triacylglycerols Containing Ximenynic Acid in *Santalum album* (Linn.) Seed Lipids

THEORETICAL

The seed lipids of a number of santalaceae species including some other plant families are rich in acetylenic fatty acids. At least one of the following acetylenic acid has been found in the seed lipids of every species of the santalaceae family that has been examined so far.

$$CH_3-(CH_2)_7-C\equiv C-(CH_2)_7-COOH$$

Octadec-9-ynoic (stearolic) acid

$$CH_3-(CH_2)_5-CH=CH-C\equiv C-(CH_2)_7-COOH$$

Octadec-*trans*-11-en-9-ynoic (ximenynic) acid

$$CH_3-(CH_2)_4-C\equiv C-CH_2-CH=CH-(CH_2)_7-COOH$$

Octadec-*cis*-9-en-12-ynoic (crepenynic) acid

$$CH_3-(CH_2)_3-CH=CH-C\equiv C-(CH_2)_7-COOH$$

Hexadec-*trans*-11-en-9-ynoic (exocarpic) acid

$$CH_3-(CH_2)_5-CH=CH-C\equiv C-(CH_2)_6-COOH$$

Heptadec-*trans*-10-en-8-ynoic (pyrulic) acid

$$CH_2=CH-(CH_2)_4-CH=CH-C\equiv C-(CH_2)_7-COOH$$

Octadec-*trans*-11,17-dien-9-ynoic acid

$$CH_2=CH-(CH_2)_4-CH=CH-C\equiv C-\underset{\underset{OH}{|}}{CH}-(CH_2)_6-COOH$$

8-Hydroxyheptadeca-*trans*-11,17-dien-9-ynoic acid

Despite the abundant occurrence of these triacylglycerols in the seed lipids of some plant species, virtually no work has been carried out to analyze the triacylglycerols containing acetylenic fatty acyl moieties. In the seed lipids of *Crepis rubra*[1], the crepenynoyl moieties were found to be esterified chiefly, but not entirely at *sn*-2 and 3 positions of the glycerol moiety.

Recently, a number of acetylenic fatty acids have been found to interfere with lipid and fatty acid metabolism and inhibit the activities of cyclooxygenase and lipoxygenase enzymes in a variety of tissues[2,3]. The 5,8,11,14-eicosatetraynoic acid has shown to be potent inhibitor of the activities of both cyclooxygenase and lipoxygenase[4] while the 5,8,11-eicosatriynoic acid showed selective inhibition of platelet 12-lipoxygenase as compared to cycloxygenase[5]. Downing *et al.*[6] studied the inhibition action of crepenynic acid and suggested that it had little significant effect on soybean lipoxygenase or the conversion of [^{14}C] arachidonic acid to prostaglandins by a sheep seminal vesicle preparation. It has been suggested that the acetylenic bond may act via an intermediate allene or vinyl hydroperoxide to irreversibly

bind to enzyme and deactivate it[7]. Very recently, Croft and co-workers[8] have investigated the differential inhibition of thromboxane B_{12} and leukotriene B_4 biosynthesis by crepenynic and ximenynic acids. Crepenynic acid was found to be more effective inhibitor than ximenynic acid[8].

The seed lipids of Ixiolaena brevicompta contain crepenynic acid as a major fatty acid component. This plant reported to be responsible for causing acute muscular degeneration and mortalities of sheeps in Western New South Wales and Queensland[9,10]. These mortalities are linked with the inhibitory effects of crepenynic acid on cyclooxygenase, lipoxygenase and phospholipase A_2 activities. It is well known that the arachidonic acid which is liberated from phospholipids by the action of phospholipase A_2, is a precursure of leukotrienes, thromboxanes and prostaglandins. It has been suggested by Croft et al.[8] that at the higher doses of crepenynic and ximenynic acids the biosynthesis of leukotriene B_4, thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ was partially inhibited due to the deactivation of phospholipase A_2.

The seed lipids of Santalum album (Linn.) also contain ~ 89% of ximenynic acid and the seeds are reported to be consumed by human beings. Keeping in view the aforementioned adverse physiological properties of acetylenic acids, we have developed interest to determine the molecular composition of triacylglycerols and the positional distribution of ximenynic acid in

S. album seed lipids. In the present work, efforts were made to analyze the triacylglycerols of S. album seed lipids by chromatographic and spectroscopic techniques.

EXPERIMENTAL PROCEDURES :

Materials and Methods

(i) Source of seeds

The *S. album* seeds were supplied by Sandal Research Centre, Bangalore, India.

(ii) Extraction of Seed Lipids

The kernels were separated out from the pericarp by light crushing of the seeds in a mortar. The kernels were then finely crushed alongwith 20 g of sodium sulfate/50 g of kernels. The lipids from the crushed kernels were extracted repeatedly with light petroleum ether (40-60°C) in a soxhlet apparatus on a water bath for 3 hr. The petroleum extract of the kernels was filtered by passing through sodium sulfate and kept at room temperature on anhydrous sodium sulfate for over night. Removal of petroleum ether by reduced pressure gave light yellowish oily lipids (60-65% yield).

(iii) Thin Layer Chromatography

Glass plates of the dimensions 20x20 cm were uniformyl coated (0.2 mm thick) with silica gel G (particle size 2-25μ) with the help of an applicator. Silica gel on plates was activated prior to use and stored in airtight TLC chamber.

The TLC plates were developed in the mixture of petroleum ether, diethyl ether and acetic acid (85:15:5, v/v/v) at 5°C. The analytical TLC plates were rendered visual by spraying with a 20% aqueous solution of perchloric acid and heating in an oven (~110°C) for 10 min. Preparative TLC plates were sprayed with 2',7'-dichlorofluorescein and viewed under UV light.

(iv) Preparation of Mixed Fatty Acids

The seed lipids (32.7 g) were hydrolyzed by refluxing with 120 mL of ethanolic potassium hydroxide solution for 1 hr. Ethanol was evaporated *in vaccuo* at 60°C. The potassium salts of mixed fatty acids were dissolved in excess of water and acidified with 1N HCl until the colour of the pH paper turns to red. The liberated free fatty acids were extracted repeatedly with diethyl ether. The combined extracts were dried over anhydrous sodium sulfate and evaporated *in vaccuo* to yield about 30.0 g of mixed free fatty acids. During saponification process, a gummy material was separated out, which was removed before acidification and extraction of the free fatty acids.

(v) Cold Esterification of Mixed Fatty Acids

The mixed fatty acids (1 g) were dissolved in 50 mL of absolute methanol containing 1% sulfuric acid (v/v) and kept at room temperature for over night under anhydrous condi-

tions in a nitrogen atmosphere. The resulting reaction mixture was diluted to the cloud point with excess water and then extracted repeatedly with diethyl ether. The combined ethereal layer was dried over anhydrous sodium sulfate for over night. Removal of diethyl ether under reduced pressure afforded methyl esters.

(vi) Isolation of Ximenynic Acid and Preparation of its Methyl Ester

The mixed fatty acids (30.0 g) of S. album seed lipids on crystallization from hexane at -10°C yielded crude ximenynic acid (22.4 g; m.p. 36-38°C). The crude ximenynic acid (22.4 g) on recrystallization from hexane gave pure ximenynic acid (19.7 g; m.p. obtained 39°C; reported[11]: 38.5°C). Methyl ximenynate was prepared by using cold esterification process as was used for esterification of mixed fatty acids. Methyl ximenynate was served as reference standard in GLC analysis.

(vii) Analysis of Triacylglycerols

The triacylglycerols of S. album seed lipids were fractionated into four components by multiple (4 times) development of thin layer chromatographic plates using petroleum ether, diethyl ether and acetic acid (85:15:5, v/v/v) at 5°C. The chromatograms were viewed under UV light after spraying with 2',7'-dichlorofluorescein. The bands corresponding to four components of triacylglycerols were scrapped off and

extracted with diethyl ether. The 2',7'-dichlorofluorescein was removed according to the procedure described by Christie[12]. The ethereal extracts of each component were dried in a glycerine bath at 40°C under the stream of nitrogen.

(viii) Transesterification of Total Lipids and Fractions

The mixture (8 mL) of methanol, benzene and sulfuric acid (84:10:4, v/v/v) was added to screw capped corning tubes of 15x125mm containing the lipid sample and refluxed at 80°C in a glycerine bath for 3 hr. After completion of the reaction period, excess of water was added to each tube and methyl esters were extracted with hexane (3x5 mL). The supernatant organic layer was washed twice with water. The hexane was evaporated under the stream of nitrogen in a glycerine bath at 40°C. The lipid samples were again dried in the similar manner by adding 3 mL of azeotropic mixture (chloroform, benzene and methanol, 1:1:1, v/v/v) in order to remove the moisture. After completion of the drying process, 0.2 mL of hexane was added again to each tube and flushed with nitrogen, immediately capped and kept at low temperature for chromatographic analysis.

(ix) Gas Liquid Chromatography

The qualitative and quantitative analyses of acyl moieties in total lipids and molecular species was carried out by using Tracor 540 gas chromatograph equipped with flame ionization detector, using a 10% silar (5 c.p., 80/100 Gas

Chrom. Q) glass column of 6ft x 2mm i.d. The qualitative examination was accomplished by spiking the retention times with authentic reference standards (methyl esters of the total seed lipids of ground nut, linn.). The peak areas were calculated by triangulation method. Methyl tridecanoate was used as internal standard for the quantitative estimation of triacylglycerol species.

(x) Nuclear Magnetic Resonance Spectroscopic (NMR) Analysis

NMR spectra were recorded on Varian A60D (60 MHz) Spectrometer in $CDCl_3$. Tetramethylsilane was used as reference standard and signals were reported here as parts per million from down field.

RESULTS AND DISCUSSION

The evergreen tree, S. album of the family santalaceae is well known for its popular and highly scented wood (sandal-wood) which is used extensively throughout India. The main sandal growing states in India are Karnataka and Tamil Nadu, and the total production of sandal seed is about 20,000 tonns per annum.

The seed lipids of S. album contain 50-60% drying oil which contains about 75% of ximenynic acid[13]. This fatty acid is known as ximenynic acid because it has been found in many Ximenia species. It is also known as santalbic acid because it has been found in most of the species of the plant family santalaceae.

The percentage of ximenynic acid in the seed lipids of S. album was variedly reported by various workers[11,14]. In our results we got about 89% of ximenynic acid (Table 1). During saponification process, the formation of gummy material was initially thought to be from ximenynic acid itself. But no such type of substance was obtained when the reaction was performed with pure ximenynic acid under similar conditions. This gummy material may be due to other components present in the seed lipids. This was quite judicial on the basis of acyl composition obtained by cold esterification of mixed fatty

acids (prepared by saponification process) as well, since no significant difference in the %ge of ximenynic acid is seen from the data (Table 1). The S. album seed lipids were applied alongwith triolein on the lanes of the TLC plates and developed in appropriate solvent system as described in experimental section. After visualization of the chromatogram, the triacylglycerols of S. album seed lipids showed slightly lower Rf value with that of triolein. The four components of the triacylglycerols overlapped with each other in single development as depicted in the Fig. 1.

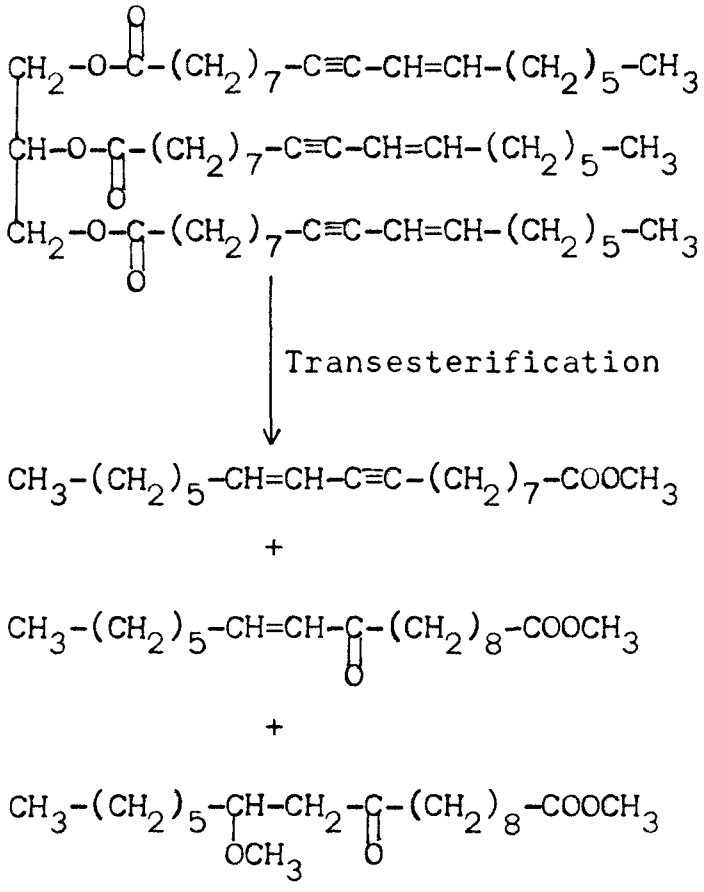
It was also reported earlier that the acetylenic fatty esters are separable from olefinic and saturated fatty esters by silica gel and gas liquid chromatography[15,16]. On this basis, we have developed the preparative thin layer chromatographic plates four times in an appropriate solvent system (Fig. 2) as described earlier. This resulted in the successful resolution of four classes of triacylglycerol species according to the number of ximenynoyl moieties i.e. triacylglycerols containing none, one, two and three ximenynoyl moieties per molecule. The absence of acetic acid (Fig. 3) in the solvent system and over loading of lipid sample on TLC plates resulted in the overlapping of triacylglycerol components. Care was taken for the quantitative analysis of these components.

After the isolation of normal triacylglycerols, monoximenynoyldiacylglycerols, diximenynoylacylglycerols and trixi-

menynoylglycerol, they were transesterified and subjected to GLC analysis. In the gas liquid chromatograms of the fractions (II, III and IV) two extraneous peaks were observed which were eluted from the GLC column after the elution of ximenynoyl moieties. The components responsible for extraneous peaks were detected by carrying a separate experiment. In this experiment, the pure triacylglycerols of S. album seed lipids were transesterified under identical conditions as those used for individual fractions. The TLC of the reaction mixture showed three products. These were separated by silica gel column chromatography. The first product (71.4%) consists of common acyl moieties and ximenynoyl moieties. The second product (4.6%) was characterized as methyl 10-oxooctadec-11-enoate. The NMR spectrum of this product showed signals at δ 6.46-7.0, m(2H, CH=CH-C(=O)-); 3.58, s(3H, -C(=O)-OCH$_3$), 2.1-2.45, m(6H, α to carbonyl groups), 0.9, t(3H, terminal methyl protons). The third product (24.0%) was characterized as methyl 10-oxo-12-methoxyoctadecanoate. The NMR spectrum of this compound showed signals at δ 3.7, s(3H, -C(=O)-OCH$_3$), δ 3.4, s(3H, -CH(OCH$_3$)-), δ 3.6, m(1H, methine) and 0.9, t(3H, terminal methyl). The spectral values of these products are identical with the α-enoate and methoxy ketones obtained by another route from methyl ximenynate[17]. Thus the components formed during acid

catalyzed transmethylation were identified and these were only responsible for extraneous peaks in GLC chromatograms. The methyl esters of the free fatty acids of S. album seed lipids prepared by cold esterification process, however, oid not show any extra peak.

Thus it is conferred that the α,β-unsaturated ketone and methoxy ketone were derived only from ximenynic acid as illustrated in the following chemical equation.



n = number of molecules

The %ge of the ximenynic acid was calculated as the sum of the peak areas due to α,β-unsaturated keto acyl, methoxy keto acyl and ximenynoyl moieties. The acyl composition of the molecular species of triacylglycerols is given in Table 2. It is apparent from this data that the triacylglycerols of S. album seed lipids are composed of four prominent types of molecular species. A group of molecular species (fraction I, 7.1%) were found to contain no ximenynoyl moieties, since these are having 88.1% of the common long-chain acyl moieties. The minor molecular species of triacylglycerols (fraction II, 1.1%) had one ximenynoyl moiety per molecule, since these are found to have 33.2% of ximenynoyl moieties in the total acyl moieties of the species. Another type of molecular species (fraction III, 3.8%) are having two ximenynoyl moieties per molecule, since these were found to be composed of 72.8% of ximenynoyl moieties. The major molecular species (fraction IV, 88%) contains three ximenynoyl moieties, since this fraction is made up of 92% of ximenynoyl moieties.

Because of the presence of exceptionally high amount of triximenynoylglycerol (88%) in the total triacylglycerols of S. album seed lipids, it was thought that there was no need to carry out the lipolysis to find out the positional distribution of ximenynic acid in the glycerol molecules. Further, the ximenynoyl moieties show inhibition of the activities of cyclooxygenase, lipoxygenase, phospholipase and prostaglandin

synthetase. Hence, it will be of interest to find out whether the same effect could be observed with lipase or not prior to the lipolysis of these triacylglycerols containing ximenynoyl moieties. Considering all these accounts, we did not perform lipolysis of the triacylglycerol components of S. album seed lipids to deduce the positional distribution of ximenynoyl moieties in the triacylglycerols.

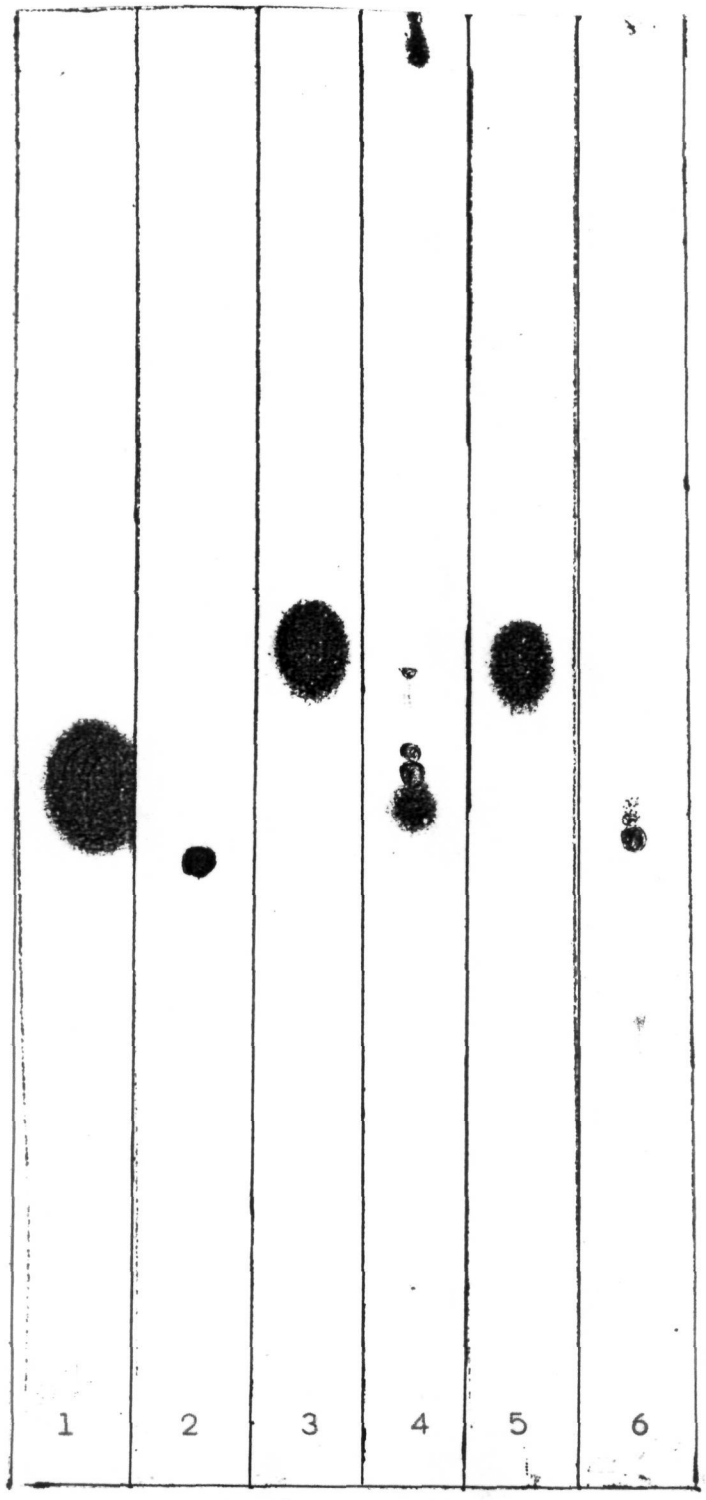


Fig. 1. Thin Layer Chromatogram of S. album seed lipids and known reference standards.

1. Oleic acid
2. Ximenynic acid.

3 and 5. Different loads of Triolein

4 and 6. Different loads of S. album seed lipids.

The plate was developed with petroleum ether, diethyl ether and acetic acid (85:15:5, v/v/v).

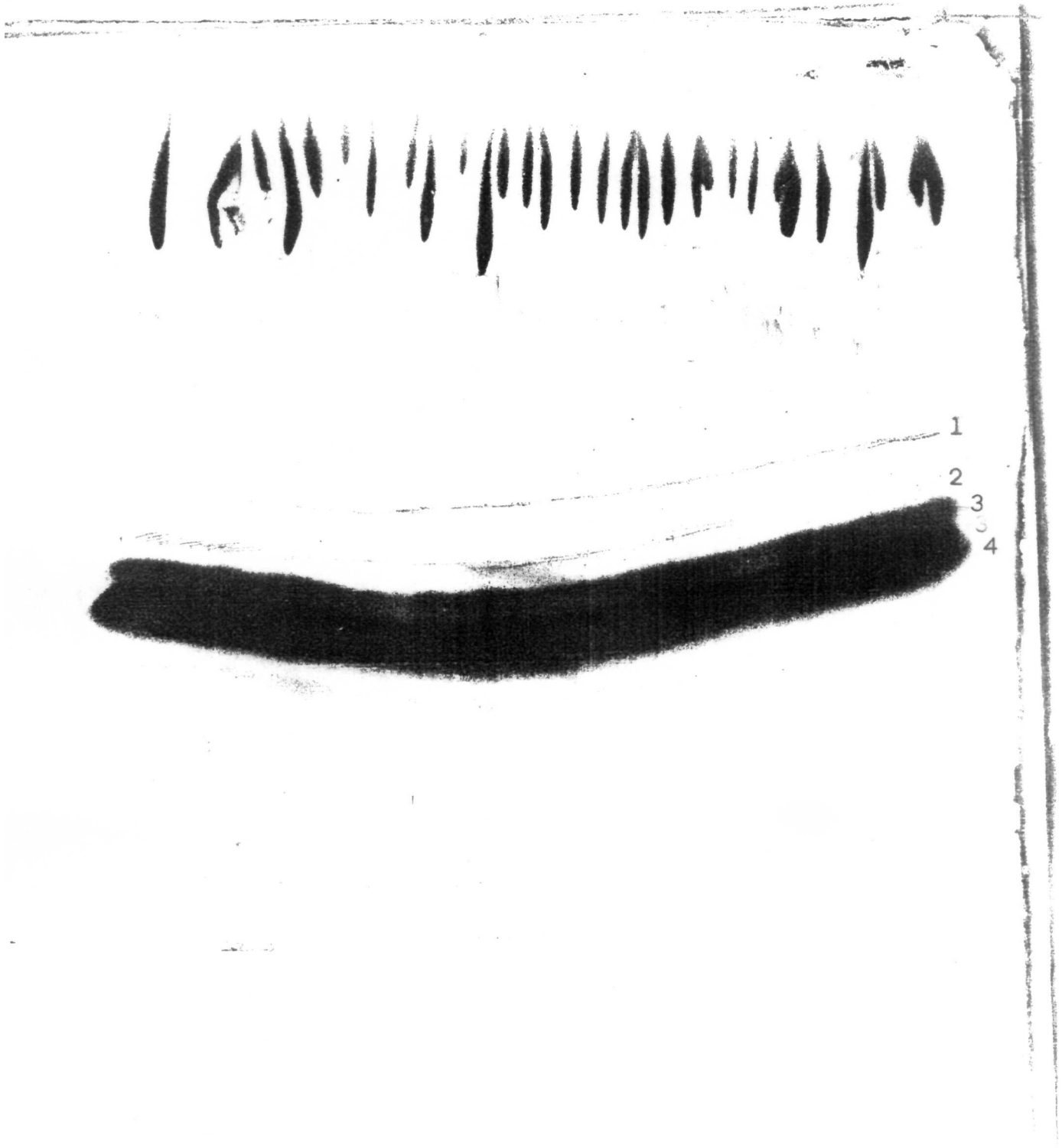


Fig.2: Separation of triacylglycerols of S. album seed lipids.

The plate was developed four times in petroleum ether, diethyl ether and acetic acid (85:15:5, v/v/v) at 5°C.

Components at the solvent front are hydrocarbons,

1. Normal triacylglycerols.
2. Monoximenynoyldiacylglycerols.
3. Diximenynoylacylglycerols.
4. Triximenynoylglycerol.

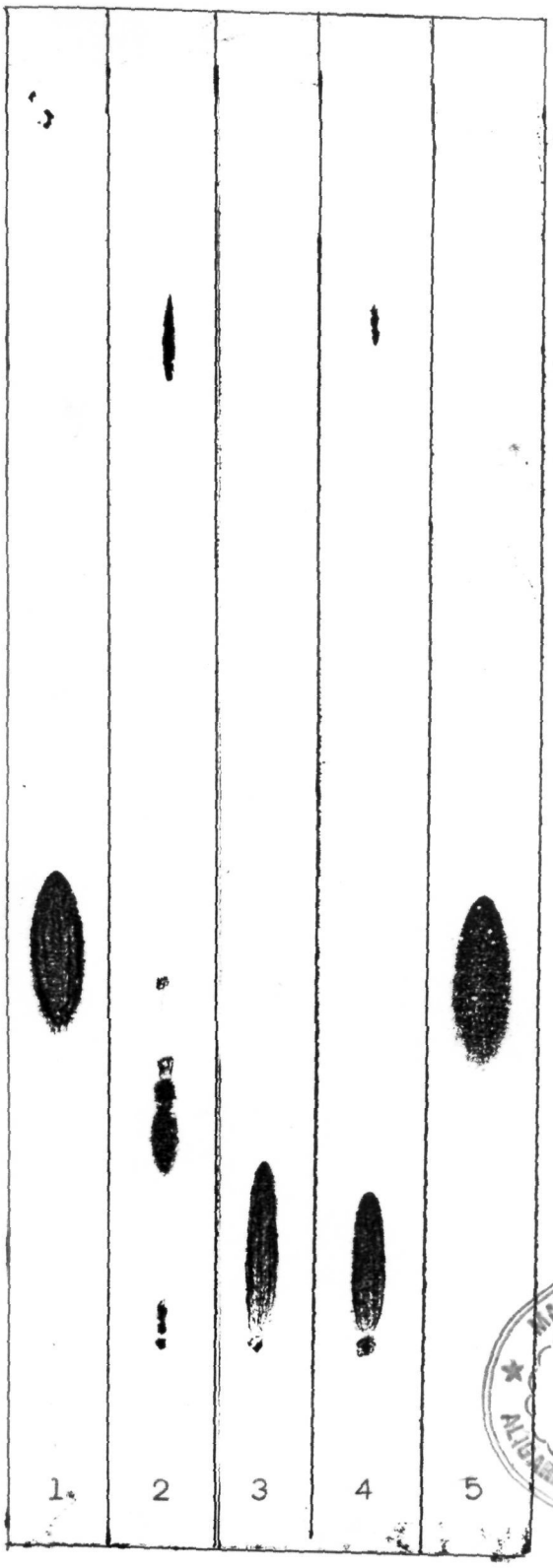


Fig. 3. Thin Layer Chromatogram of S. album seed lipids and known reference standards.

1 and 5. Different loads of Triolein

2. Seed Lipids of S. album

3. Oleic acid

4. Ximenyic acid

The plate was developed with petroleum ether and diethyl ether (85:15, v/v).

TABLE 1

Acyl composition of *Santalum album* seed lipids after methylation.

Acyl moieties[a]	Total methyl esters[b]	Total methyl esters[c]
16:0	0.8	1.2
16:1	0.2	0.2
18:0	trace	trace
18:1	7.4	8.0
18:2	0.4	0.3
18:3	0.7	1.0
18:EY	89.6	88.2[d]
Others (Unidentified)	0.9	1.0

[a]Acyl moieties are designated according to number of carbon atoms : number of double bonds; 18:EY, Ximenynoyl.

[b]Prepared by cold esterification process.

[c]Prepared by using methanol, benzene and sulphuric acid (84:10:4, v/v/v) at 90°C.

[d]Calculated as the sum of the peaks due to 18:EY and keto and methoxy keto derivatives of 18:EY.

TABLE 2

Acyl[a] composition (wt %) of molecular species of triacylglycerols in *Santalum album* seed lipids

Fraction	Relative Proportion %	16:0	16:1	18:0	18:1	18:2	18:3	18:EY	Others (unidentifie
I	7.1	7.8	4.0	trace	68.2	4.8	6	7.1[b]	2.1
II	1.1	4.4	trace	trace	62.2	-	-	33.2[b]	-
III	3.8	0.8	-	trace	17.6	trace	8.1	72.8[b]	0.6
IV	88.0	-	-	-	0.5	-	7.3	92.0[b]	0.2

[a]Acyl moieties are designated according to number of carbon atoms and number of double bonds.

[b]Calculated as the sum of the peaks due to 18:EY and keto and methoxy keto derivatives of 18:EY.

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